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Chemical, physical and glycaemic characterisation of PulseON®: A novel legume cell-powder ingredient for use in the design of functional foods

Cathrina H. Edwards[a,*], Peter Ryden[a], Ana M. Pinto[b], Alice van der Schoot[b], Costanza Stocchi[b], Natalia Perez-Moral[a], Peter J. Butterworth[b], Balazs Bajka[b], Sarah E. Berry[c], Sandra E. Hill[d], Peter R. Ellis[b]

[a] Quadram Institute Bioscience, Norwich Research Park, Colney, Norwich NR4 7UQ, United Kingdom
[b] Biopolymers Group, Departments of Biochemistry and Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom
[c] Diet and Cardiometabolic Group, Department of Nutritional Sciences, Faculty of Life Sciences and Medicine, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom
[d] Biopolymer Solutions Ltd., College Road, Sutton Bonington, Loughborough LE12 5RD, United Kingdom

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ABSTRACT

Pulses are high in protein and dietary fibre but their long preparation time can be a barrier to consumption. We have developed PulseON® – novel pre-cooked cell powders that can be prepared from various legumes and used as functional food ingredients. Techno-functional characteristics and starch digestibility of powders prepared from seven different pulses were compared to flours from the same source. All PulseON® powders consisted of intact plant cells with low starch digestibility (< 40% starch digested at 90 min) compared with cooked pulse flours (> 80% starch digested within 30 min) and had a higher water holding capacity and swelling power than their flour counterpart. A glycaemic study in healthy human subjects demonstrated that the chickpea PulseON® had a low-medium glycaemic index. Overall, PulseON® powders provide superior starch resistance to normal pulse flours and their glycaemic properties show promise in functional food applications to benefit cardiometabolic health.

1. Introduction

Pulses are dry non-oilseed grains of the *Leguminosae* family and have been identified by The Food and Agriculture Organisation of the United Nations (FAO) as an important crop for food security owing to their agricultural and nutritional properties (Calles, Xipsiti, & del Castello, 2019). Regular intake of pulses is associated with lower body weight and improved markers of glycaemic control and lipid metabolism (Krug, 2016; Reynolds et al., 2019; Willett et al., 2019), while the acute beneficial effects of pulses on postprandial satiety and glycaemia are widely reported (Jenkins, Wolever, Taylor, Barker, & Fielden, 1980; Mollard et al., 2012). Furthermore, the low Glycaemic Index (GI) of pulses has been shown to benefit dietary prevention and management of Type 2 diabetes (Sievenpiper et al., 2009), which is currently one of the top ten leading causes of death worldwide (World Health Organisation, 2016). Despite this, pulses are underutilised. On average, the UK population consumes less than 2.5 g of beans and pulses per person per day (Roberts, Steer, Maplethorpe, Cox, Meadows, Nicholson, & Swan, 2018) and intakes are also declining in many African countries where pulses were traditionally a staple food (Mattei et al., 2015). Consumers report long preparation time as a barrier to consumption, hence increasing the use of pulse-derived ingredients in processed foods is a strategy to increase dietary intakes.

Pulses are generally processed by splitting, milling and/or fractionation before use as food ingredients, and are already widely used in gluten-free product applications and other applications where their functional properties (water holding, swelling power, gelatinisation, etc.) enhance product formulation (Foschia, Horstmann, Arendt, & Zannini, 2017). Compared to plain white wheat flour, pulse flours contain approximately twice as much protein and dietary fibre, so incorporation of pulse flours into cereal-based products can also improve nutrient profiles of food products, to support the health of the general

* Corresponding author.
E-mail addresses: cathrina.edwards@quadram.ac.uk (C.H. Edwards), peter.ryden@quadram.ac.uk (P. Ryden), ana_margarida.pinto@kcl.ac.uk (A.M. Pinto), alice.van_der_schoot@kcl.ac.uk (A. van der Schoot), costanza.stocchi@kcl.ac.uk (C. Stocchi), natalia.perez@quadram.ac.uk (N. Perez-Moral), peter.butterworth@kcl.ac.uk (P.J. Butterworth), balazs.bajka@kcl.ac.uk (B. Bajka), sarah.e.berry@kcl.ac.uk (S.E. Berry), sandra.hill@biopolymersolutions.co.uk (S.E. Hill), peter.r.ellis@kcl.ac.uk (P.R. Ellis).

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public (Jukanti, Gaur, Gowda, & Chibbar, 2012).

Pulses are also a potential source of Type 1 resistant starch (RS1), which is present within intact plant cells wherein the plant cell wall ('dietary fibre') encapsulates the starch and greatly reduces its accessibility to digestive amylases in the gastrointestinal lumen (Berg, Singh, Hardacre, & Boland, 2012; Bhattarai, Dhital, Wu, Chen, & Gidley, 2017; Dhital, Bhattarai, Gorham, & Gidley, 2016; Golay et al., 1986; Grundy et al., 2016; Würsch, Del Vedovo, & Koellreutter, 1986). Leguminous cells can remain intact in whole boiled pulses or in other foods, where the seeds have been prepared under hydrothermal conditions. Whole or partial solubilisation of the pectin in the middle-lamella enables cell separation during subsequent mechanical disruption (Melito & Tovar, 1995; Tovar, Björck, & Asp, 1992). After consumption of whole boiled pulses, cells containing entrapped starch have been identified in masticated expectorate (Pallares Pallares, Loosveldt, Karimi, Hendrickx, & Grauwet, 2019) and in the small intestinal fluid of humans (Dhital et al., 2016; Noah et al., 1998). The limited bioaccessibility of starch from intact cotyledon cells of pulses means that the postprandial glycaemic and insulinaemic responses are attenuated (Golay et al., 1986; Tovar, Granfeldt, & Björck, 1992), while subsequent fermentation of carbohydrates by the colonic microbiota produces metabolites associated with colonic health benefits (Kendall, Emam, Augustin, & Jenkins, 2004; Warren et al., 2018). However, most processing methods that are currently used to prepare pulse flours, such as dry-milling, compromise cell structure so that products made from pulse flours do not retain the RS1 nor the associated low glycaemic properties (Pallares Pallares et al., 2018, 2019; Verkempinck, Pallares Pallares, Hendrickx, & Grauwet, 2019).

Development of alternative pulse flours with a higher proportion of intact cells is therefore an area of emerging interest (Anderson et al., 2014; Boukid et al., 2019; Tosh et al., 2013). We previously reported that preserving a larger proportion of intact cells within macro-milled particles was associated with reduced starch digestibility (Edwards, Maillot, Parker, & Warren, 2018; Edwards, Warren, Milligan, Butterworth, & Ellis, 2014), and this approach was recently used by Boukid *et al.* to produce macro-particles with intact cells for use in bread applications (Boukid et al., 2019). Tosh *et al.* have developed spray-dried cellular legume powders with ~5% resistant starch (RS) (Tosh et al., 2013), but the level of RS in their powders does not seem to be sufficient to produce significant effects on glycaemic control (Cryne et al., 2012). While another study reported no difference in blood glucose responses between canned and powdered pulses (Anderson et al., 2014), there is convincing evidence that the processing conditions used to prepare whole pulses is an important factor that affects subsequent starch resistance (Pallares Pallares et al., 2019).

We have developed a novel method of processing pulses into a cellular powder, PulseON®, and recently demonstrated that incorporation of chickpea PulseON® lowers the *in vitro* starch digestibility of savoury wheat biscuits (Delamare et al., 2020). In the present study, we test the hypothesis that these powders contain high levels of type 1 RS and elicit an attenuated glycaemic response. The aim of this study was to determine the techno-functional characteristics, starch digestibility and *in vivo* glycaemic response to these powders. Here, we describe the characteristics of these novel pulse powders and compare their properties to dry-milled pulse flours. The ingredients described are well aligned to current market trends for plant-based eating and consumer preference towards convenience food products. Also, the reported findings provide new opportunities for aiding formulation of healthier pulse-based products for these emerging markets.

2. Materials & methods

2.1. Food materials

Whole raw Kabuli chickpeas (*Cicer arietinum* L., Russian cv.), hereafter known as CP, were obtained from AGT Poortman (London, UK).

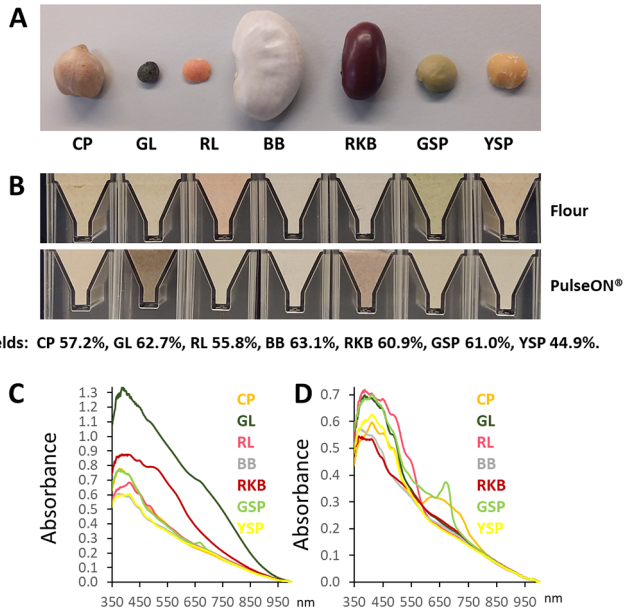

Fig. 1. Physical appearance of whole pulses and flour and PulseON® powders derived from these. Photograph showing physical appearance of whole pulses (A) and the appearance of dry-milled pulse flours and PulseON® powders, including the yields for PulseON® powders obtained from each pulse source (B). A difference in colour can be seen between powders and flours obtained from the same source. Notably, PulseON® from GL and RKB have darker brown hues compared with the flour from the same source, whereas PulseON® from RL, GSP and YSP have paler colours than their flour counterparts. Reflectance measurement of PulseON® powders (C) and flour (D) obtained from different pulses; chickpea (CP), green lentils (GL), red lentils (RL), butterbeans (BB), red kidney beans (RKB), green split peas (GSP), and yellow split peas (YSP). Readers should note the difference in scale on the y-axis (C and D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Other pulses were obtained from a local supermarket (Sainsbury's, UK); *Lens culinaris* 'green lentils' (GL) and 'red lentils' (RL), *Phaseolus lunatus* 'butter beans' (BB), *Phaseolus vulgaris* 'red kidney beans' (RKB), *Pisum sativum* 'green split peas' (GSP) and 'yellow split peas' (YSP). The seed coats (testa) were present on the pulses CP, GL, BB and RKB, whereas the RL, GSP, and YSP were supplied as de-hulled seeds. The physical appearance and nutrient composition of the whole seeds is shown in Fig. 1 and OSM 1, respectively.

These dry seeds were processed into novel cellular powders through a proprietary process as described in detail elsewhere (PCT/GB2019/050284) and involved hydrothermal treatment of soaked seeds in water at 100 °C for 90 min (CP), 50 min (GSP), 30 min (RKB), 25 min (YSP and GL), or 15 min (RL and BB), and subsequent wet-sieving to harvest the cells (< 150 μm), followed by air-drying to obtain a powder with moisture content < 6% (Edwards, Ellis, et al., 2019). The differences in hydrothermal treatment times reflect the different cooking conditions required to achieve high levels of cell separation for individual pulses. The yield was calculated as the % of original dry matter (DM) recovered in the cellular size fraction.

For comparison, dry-milled flours were produced from the same batch of pulses. Those pulses with a testa (CP, GL, BB, RKB) were soaked in water (50 g in 400 mL) for 3–6 h, each testa was peeled off, and then the pulses were dried at 44 °C for 16 h. The dehulled pulses were all blended in a KRUPS F20342 Coffee Grinder for 1 min in six periods of 10 s with a pause of 5 s in between each period. The milled material was sieved on a 150 μm Endecott sieve to obtain a sub-cellular flour showing a high degree of cell rupture as would occur during commercial milling. The oversized particles were re-blended for up to 3 min and then re-sieved.

2.2. Proximate composition of ingredients

Proximate analyses of the seven PulseON® powders and pulse flours were performed by UKAS accrediting testing at ALS laboratories UK Ltd. at Chatteris, Cambridgeshire, UK. Protein was determined by Dumas Nitrogen using a conversion factor of 6.25, total fat by NMR, total dietary fibre by AOAC, total sugars by ion-exchange HPLC, sodium by ICP-OES, and 'available' carbohydrate calculated 'by difference' unless otherwise specified. Energy values were calculated using standard conversion factors.

2.3. Total starch and moisture determination

Direct measurement of total starch was performed according to the AOAC 996.11 Method (DMSO format) using enzymes and reagents supplied by Megazyme International (Total Starch assay kit K-TSTA) and performed at 1/10th scale as suggested by the suppliers. Powders and flours were ground with pestle and mortar and analysed as reported previously (Edwards et al., 2015). Data was expressed on a dry weight basis. Briefly, samples (about 100 mg) were weighed in 35 mm diameter aluminium pots and dried at 103 °C in a vented oven (Binder Model ED-56) over night. Sealed pots were cooled for 30 min in a desiccator over 3 Å molecular sieve, then weighed. The moisture content was calculated as percent water loss.

2.3.1. Water holding capacity

The water holding capacity of the pulse powders (particle size 106–250 μm) and the dry-milled flours were determined with a modified version of the AACC method no. 51-61 (AACC, 1990). In brief, 1:5 (w/v) flour or powder to water suspensions were centrifuged for 15 min at 1000*g* at 20 °C and the resulting supernatant was removed, and the remaining wet pellet was weighed. The water holding capacity was calculated from the mass of water absorbed divided by the mass of dry flour or powder.

2.3.2. Swelling power

For assessment of swelling power, 1:30 (w/v) ratio flour or powder to water suspensions were vortexed to suspend the flour/powder and then heated in a Thermomix block for 30 min with rotation at 1400 rpm. Flours were heated at 37, 60, 70, 80 and 95 °C and powders were heated at 37, 60 and 95 °C. The tubes were cooled for 5 min then centrifuged at 13,000*g* for 10 min, the supernatant aspirated, and the wet pellet weighed. Swelling power was calculated as the mass of wet pellet divided by the mass of dry matter, and the resulting value includes the water bound to the external particle surfaces.

2.4. Microscopy

Light micrographs were captured with an Olympus BX60 Microscope equipped with Jenoptik ProgRes camera and a ProgRes CapturePro software. For polarised microscopy, birefringence was assessed by viewing samples on the microscope fitted with crossed polarisers. Samples were viewed 'as is' (without prior fixation) and some specimens were stained with Lugol's Iodine (I_2/KI) solution (Sigma Aldrich Ltd., UK).

2.5. Colour by reflectance

Flours and powders were packed into crimp caps. The probe was placed within approximately 2 mm of the surface of the probe of a Stellarnet VIS/NIR system. A white teflon bar provided the diffuse reflectance standard, and 100 scans of the absorbances were obtained with a detector integration time of 30 ms. The data were saved after the screen had refreshed twice. The spectra were truncated to 350–1000 nm and baseline anchored at 1000 nm.

2.6. Particle size

The particle size distribution was measured by laser-light diffraction (Beckman LS 13320 with the Universal Liquid Module). The optical parameters chosen were a particle and dispersant (water) refractive index of 1.456 and 1.330, respectively. About 50 mg of each flour was dispersed in 1 mL of water and the particle size was measured. The size distribution was obtained using polydisperse analysis, as surface area weighted ($d_{3,2}$) means, where $d_{3,2}$ is defined as $\Sigma n_i d_i^3 / n_i d_i^2$, where n_i is the number of particles with diameter d_i. Each measurement was carried out in triplicate.

2.7. Differential scanning calorimetry (DSC)

The DSC thermograms were recorded using a differential scanning calorimeter (Mettler Toledo, DSC3+, Leicester UK). Approximately 4 mg of flours (all of similar particle size) or PulseON® powders were weighed into stainless steel pans and water was added at a ratio of water to solids of 3:1. The pans were sealed and an empty steel pan was used as a reference sample. The samples were heated from 20 °C to 150 °C at 10 °C/min. Different peaks associated with thermal transitions occurring in the sample were monitored by using STARe Thermal Analysis software. Onset, peak and conclusion temperatures (denoted *To, Tp, Tc*) and the enthalpy changes (ΔH) were obtained from each thermogram as described elsewhere (Bogracheva, Wang, Wang, & Hedley, 2002).

2.8. X-ray diffraction (XRD)

Pulse flours and cell powder samples in randomly oriented forms were packed into disc shaped plastic sample holders, which were mounted in a Bruker D8 Advance Eco X-ray diffractometer set up in a slit focus θ/θ reflection geometry mode. Copper K alpha (Cu Kα) radiation of wavelength 1.5418 Å was generated by an x-ray tube at voltage and current settings of 40 kV and 40 mA. Wide angle measurements were carried out in the range 4–45° 2θ.

2.9. In vitro starch digestibility assay

The details of the digestibility method and principles of starch amylolysis and relevance to prediction of glycaemic responses have been described elsewhere (Butterworth, Warren, Grassby, Patel, & Ellis, 2012; Edwards et al., 2014; Edwards, Cochetel, Setterfield, Perez-Moral, & Warren, 2019; Goñi, Garcia-Alonso, & Saura-Calixto, 1997). In brief, ~90 mg of pulse powder or flour were weighed into 15 mL Falcon tubes and suspended in 10 mL of phosphate buffered saline (PBS, Oxoid, pH 7.4 at 37 °C) by vortex mixing. The suspensions were put in the platform (prs 26) of a PTR-35 Grant-bio rotator at the appropriate spacing for simultaneous sampling of 3 tubes with a multi-pipette. For equilibration, the suspensions were mixed end-over-end at 60 rpm inside a 37 °C incubator for 15 min (E24 Excella, New Brunswick Scientific). A dilution of porcine pancreatic α-amylase (EC 3.2.1.1 A6255, Sigma-Aldrich) was prepared in phosphate buffered saline (PBS) to obtain a working solution with amylase activity of 89 U/mL. The rotation was halted to allow the samples to settle for 15 s, then aliquots of 100 μL were withdrawn and stopped in an equal volume of 0.3 M Na_2CO_3. Triplicate additions of amylase were made at 15 s intervals to start the digestion. The digestions were sampled at 10, 20, 30, 40, 50, 60, 75, 90 and 120 min. The stopped aliquots were centrifuged at 15,000*g* for 5 min at 20 °C (Heraeus Pico, Thermo Scientific) and 130 μL of the supernatants were retained. Samples collected during starch amylolysis were diluted with deionised water and analysed by the PAHBAH assay as described previously (Edwards et al., 2018, Edwards, Cochetel, et al., 2019) to determine the concentration of reducing sugars (as maltose equivalents) at each time point and expressed as the percentage (%) of starch digested. In this study, the incubation mixture

Table 1
Proximate composition (per 100 g ingredient 'as is') of dry-milled flours and PulseON® powders from different pulses.[1]

	Pulse Flours							PulseON® powders						
	CP	RL	GL	BB	RKB	GSP	YSP	CP	RL	GL	BB	RKB	GSP	YSP
Energy (KJ)	1547	1455	1498	1408	1421	1430	1406	1462	1345	1363	1326	1334	1321	1350
Energy (kcal)	367	343	354	333	336	338	332	349	318	322	315	316	313	320
Fat (g)	6.4	0.8	1.1	0.9	1.4	1.2	1.0	6.5	0.7	0.6	0.9	1.0	0.8	1.0
Avail. CHO (g)	55.9	55.7	54.6	52.6	52.7	55.6	57.2	50.0	48.8	48.9	43.4	46.8	45.1	52.6
Sugars (g)	3.1	1.6	1.1	5.5	3.9	3.1	2.5	2.0	0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1
Starch (g)	52.8	54.0	53.5	47.2	48.8	52.5	54.7	48.0*	48.6	48.9	43.2	46.8	45.1	52.6
Dietary fibre (g)	8.6	6.1	6.2	9.5	12.6	8.1	8.4	19.9	13.2	12.3	18.4	18.3	20.1	15.1
Protein (g)	17.1	25.3	28.2	23.8	21.9	22.2	19.4	19.5	22.7	24.2	24.0	20.9	21.4	17.5
Sodium (mg)	< 3	< 3	< 3	< 3	< 3	< 3	< 3	34.9	11.5	10.9	12.5	12.7	9.99	11.3
Moisture (g)	9.3	9.9	7.5	9.0	7.8	10.7	11.4	3.6	13.8	12.9	12.4	11.9	11.5	12.4

[1] Values determined using accredited test methods by ALS (UK) Ltd.' Fat was determined by NMR, protein using N × 6.25, sugars by ion-exchange HPLC, sodium by ICP-OES and dietary fibre by AOAC. Available carbohydrates (Avail. CHO) were calculated by difference, and for sample marked *direct enzymatic determination was used to determine starch content. Ingredients were obtained from different pulses; chickpea (CP), green lentils (GL), red lentils (RL), butterbeans (BB), red kidney beans (RKB), green split peas (GSP), and yellow split peas (YSP).

contained 0.89 U amylase/mL with ~4.5 mg/mL starch (~0.2 U/mg starch) where 1 U is defined as the amount of enzyme required to liberate 1.0 mg maltose from soluble potato starch in 3 min at pH 6.9 and 20 °C.

2.10. In vivo glycaemic responses

A human study was performed to determine the glycaemic response to PulseON® made from chickpeas. The study took place between June and September 2019 and the protocol was registered at clinicaltrials.gov as NCT03994276. This study was conducted according to guidelines laid down in the Declaration of Helsinki and approved by the relevant ethics committee in the United Kingdom (BDM Research Ethics Subcommittee, King's College London, HR-18/19-8431). All volunteers gave their written informed consent after being provided with oral and written information about the aims and protocol of the study.

Healthy non-smoking participants aged 18–45 years were recruited through internal circular emails and flyers at King's College London. Participants were excluded if their body mass index (BMI) was < 18 or ≥35 kg/m^2, seated blood pressure ≥160/100 mmHg, fasted glucose > 6.0 mmol/L, plasma cholesterol ≥7.8 mmol/L, plasma triacylglycerol ≥5.0 mmol/L, suffered from diabetes or phenylketonuria, had a history of cardiovascular or kidney disease, cancer, chronic liver disease or were taking medication for any chronic medical conditions, or were pregnant, breastfeeding, or intolerant or allergic to any of the study foods. BMI, blood pressure and fasting blood glucose, blood lipids, liver function and full blood counts were assessed at a screening visit to confirm eligibility before enrolment onto the study. For each study visit, participants attended the Metabolic Research Unit at King's College London, UK after an overnight 12 h fast. At each visit, the participants received a test drink containing 58 g available carbohydrate, either in the form of glucose (oral glucose tolerance test, using dextrose powder from Thorton & Ross, England) or as chickpea PulseON®, where 50 g of available carbohydrate was provided by the chickpea powder (100 g powder) and 8 g of available carbohydrates provided by chocolate flavouring (11 g Nesquik, chocolate flavour, no added sugar, added to aid palatability). To ensure the test carbohydrates remained in solution, all test drinks were prepared in an equivalent volume of 330 mL (bottled water, Tesco Ashbeck). Drinks were consumed in random order on separate visits at least 3 days apart, and all drinks were consumed within 8 min. On each occasion, blood glucose concentrations were measured in capillary whole blood obtained by finger prick using an Accu-Check® Performa nano device with test strips (Roche Diabetes Care Australia Pty. Ltd) and lancets (GlucoRx, Surrey, UK). Blood glucose was measured at baseline immediately before consumption of the test meal (fasting, t = 0), and at 10, 20, 30, 45, 60, 90, and 120 min thereafter.

The glucose response curves obtained were plotted in Graph Pad Prism 8 software for each participant, and these curves were used for subsequent data analysis. The incremental area under the blood glucose response curves 0–120 min (iAUC) to test and reference drinks were calculated geometrically using the trapezoid rule, ignoring the area below the fasting baseline. For each test food, the iAUC was expressed as a percentage of the iAUC for the *iso*-carbohydrate reference drink (glucose) consumed by the same participant. The GI of each food was then calculated as the mean value across all participants consuming that food. The glycaemic testing protocol used in this study is consistent with recommendations for test procedures described elsewhere (Brouns et al., 2008).

3. Results

3.1. Physico-chemical characteristics of pulse powders

The yield of PulseON® powder from each of the different pulses varied from 45% (YSP) to 63% (BB), and all values are shown in Fig. 1. The texture of the boiled pulses was also observed to vary between species; BBs were firm, CPs, RKBs and GSPs were fairly firm, while lentils (RL and GL) and YSPs were soft, and although texture analysis was not included in the present study, it may be that textural differences provide some indication of the likely yield of cell powder. The pulse powders obtained from beans, chickpeas, peas, and lentils varied in colour from pale white (BB and YSP), yellow (RL, CP, GSP), and red to brown (RKB and GL), as seen in the photographs and reflectance measurements and shown in Fig. 1B and C. The colour profiles obtained for flour equivalents were less diverse (Fig. 1D).

The proximate composition of PulseON® powders compared to dry-milled flours from the same source is shown in Table 1. PulseON® powders contained up to 2.3 times more dietary fibre compared with the flours obtained from the same source. This difference is most likely attributed to the higher proportion of resistant starch (particularly Type 1) present in the PulseON® samples. It is also noteworthy that the chickpea samples had a considerably higher fat content compared with the other pulses.

Light micrographs confirmed that the PulseON® powders consist predominantly of intact cellular structures, where the starch and protein are encapsulated within plant cell walls (Fig. 2A). There was minimal free starch, intracellular debris or testa present. Polarised light micrographs (Fig. 2B) reveal evidence of birefringence and is indicative of ordered structures (e.g., starch) still present within the cellular powders. Light micrographs of cooked pulse flours on the other hand consist of ruptured cells, with exposed gelatinised starch granules

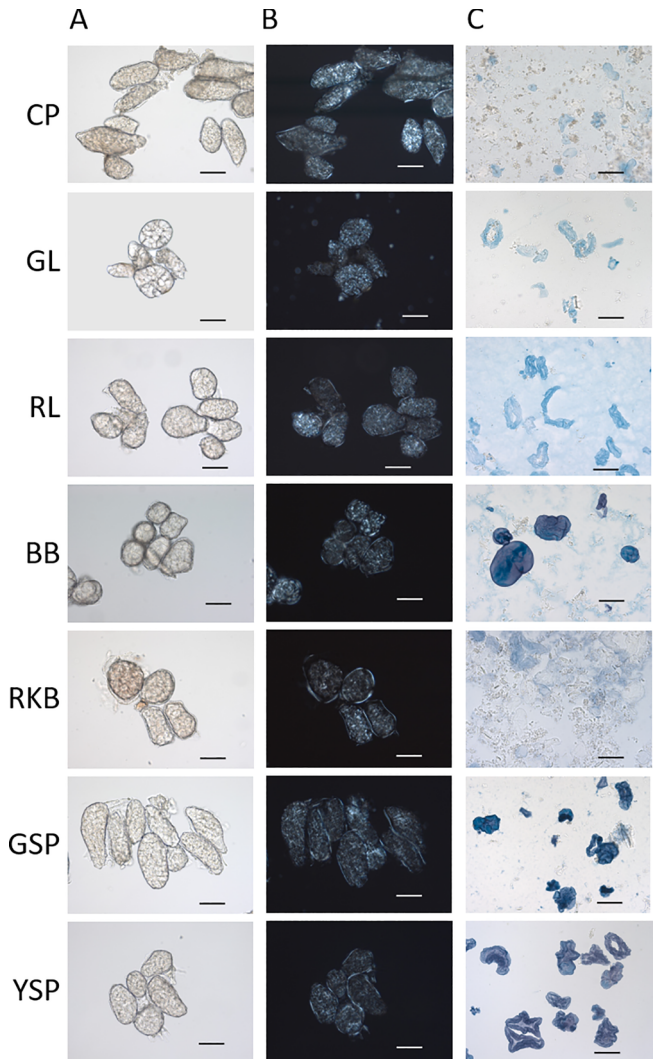

Fig. 2. Light micrographs of pulse ingredients. Micrographs of PulseON® powders (A), polarised view of PulseON® powders (B) and boiled flours stained with Lugol's Iodine (C), obtained from different pulses; chickpea (CP), green lentils (GL), red lentils (RL), butterbeans (BB), red kidney beans (RKB), green split peas (GSP), yellow split peas (YSP). Scale bar = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2C). Thus, an important difference between pulse powders and flours is that the latter consist of ruptured cells and cell wall fragments.

These microstructural characteristics of the pulse powders are reflected in the particle size distributions (Fig. 3) obtained for each pulse powder. Fig. 3 also includes a table insert with the percentiles Dv10, Dv50 and Dv90 that describe size distribution obtained for all materials. Pulse flours had a bimodal distribution, with a first peak in the region 20 to 36 μm, depending on the botanical source, and a second peak around 140 μm. Flour particles within the first peak are likely to be individual starch granules, whereas the larger particles that constitute the second peak seemed to be tissue fragments. PulseON® showed a unimodal distribution, with a single peak at ~200 μm (assuming a spherical diameter), and particles within this size range (based on a spherical diameter) consisted of cellular materials only.

3.2. Hydration characteristics

The water holding capacity (WHC) and swelling power (SP) of powders and flours obtained from the different pulses is shown in Fig. 4. PulseON® powders had a higher water holding capacity (WHC measured at 20 °C) than flours from the same botanical source. The measured values were fairly similar across all botanical sources, with the exception of RKB flour, which had a higher WHC than the other pulse flours. Most of the pulse powders held approximately twice as much water as the flour equivalent (average WHC 2.1 g water/g flour vs. 4.1 g water/g powder), with the exception RKB, where the WHC of the flour was higher than the other pulse flours (WHC of RKB flour = 3.2 g water/g flour).

SP was measured after application of several different incubation temperatures that ranged from 37 to 95 °C. The SP of PulseON® powders ranged from 5.35 to 7.00 g water/g powder and was stable across this entire temperature range. In contrast, the SP of pulse flours ranged from 2.05 to 7.48 g water/g flour in a temperature dependent manner. Thus, the SP of flours at lower temperatures was lower than that of powders, but after hydrothermal treatment, the flours and PulseON® powders had a similar swelling power. For most pulse flours the onset of swelling was detected at temperature above 60 °C, but for BB the increase in swelling was not detected until the temperature had reached 80 °C.

3.3. Thermal properties and crystallinity

Enthalpy parameters obtained from all pulse flours and PulseON® powders are shown in Table 2. It could be assumed that starch gelatinisation in pulse flours occurred over a similar temperature range (61–85 °C) for most pulse flours, except for BB, which had a higher onset, peak and concluding temperature of gelatinisation (80.3, 86.1 and 96.8 °C, respectively) compared with the other pulses (Fig. 5A1). The first gelatinisation peak for the PulseON® powders occurred at a lower temperature than flours from the same botanical source (average T_o, T_p, T_c values with SD were 51.9 ± 0.2, 61.3 ± 0.2 and 73.5 ± 0.2 °C for PulseON® powders and 65.2 ± 0.2, 73.8 ± 0.1, and 84.3 ± 0.6 °C for flours, respectively). The average enthalpy of gelatinisation in all flour samples was 6.4 J/g and ranged from 3.6 to 9.0 J/g sample, whereas the average enthalpy was 3.6 J/g for PulseON® powders and ranged from 2.9 to 4.7 J/g sample. After the DSC measurement for the flours the DSC pans were kept for at least 7 days at 4 °C. These pans were then rerun in the DSC. Enthalpies occurred at temperatures and amounts similar to those obtained for the PulseON powders (example data Fig. 5A2) and could therefore be expected to reflect a significant amount of retrogradation occurring in the processed pulses.

Protein denaturation could also give rise to enthalpic events. Raw flours CP, RKB, RL and BB showed a second enthalpy with the peak occurring between 80 and 100 °C. These temperatures are in line with those observed for isolated legume proteins when measured at similar moisture contents (Ladjal-Ettoumi, Boudries, Chibane, & Romero, 2016). For the CP and RL flours the enthalpies of the high temperature peak were small (< 0.4 J/g of sample), while it was ~1 J/g for the RKB and largest for BB, although the second peak for BB could not be reliably integrated due to overlap with the first peak (see examples in Fig. 5A1). Similarly, a second high temperature melting endotherm was also observed around 84 °C for PulseON® powders, but these peaks occurred with GSP and YSP, with enthalpies 0.13 J/g sample, and these samples did not show peaks with the raw flours. For CP PulseON® a peak did occur around 90 °C, with an enthalpy of ~1.3 J/g sample *(data not shown)* and was therefore much greater than that observed for the raw flour. The enthalpies would therefore seem to be dependent on the composition of the samples and their prior hydrothermal history.

The high levels of organisation, as shown by their X-ray diffraction, was apparent for all the flours, but is still present in the PulseON® powders. Fig. 5B shows examples of the X-ray patterns for the chickpea flour and the corresponding PulseON® powder and the difference between the patterns of these materials was representative of results

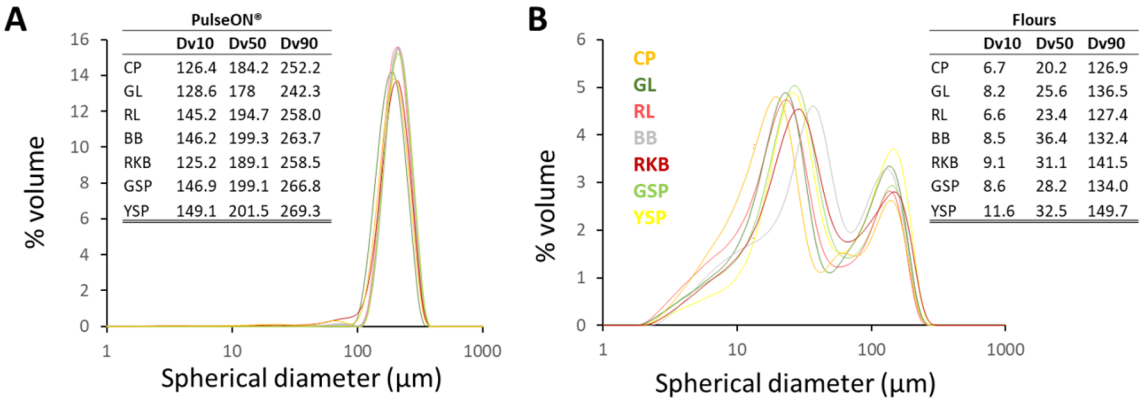

Fig. 3. Particle size distributions of PulseON® cell powders (A) and flours (B). Mean of triplicates. Legend applies to both panels. Table insert shows values of Dv10, Dv50 and Dv90, defined as the maximum particle diameter (µm) below which 10, 50 and 90% of the sample volume exists. Materials were obtained from the following pulses; chickpea (CP), green lentils (GL), red lentils (RL), butterbeans (BB), red kidney beans (RKB), green split peas (GSP), and yellow split peas (YSP). Note that in these calculations the particles are taken to be spherical. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

obtained with the other pulses. There were no obvious signs of native starch remaining in the PulseON® powders, but still considerable order and amylose–lipid peaks were seen (Becker, Hill, & Mitchell, 2001).

3.4. Starch digestibility and glycaemic responses

For the starch digestibility assays and calculation of predicted GI, the amount of material tested was adjusted so that the amount of total starch reacted with amylase was kept constant. The total amount of starch was determined directly for all PulseON® powders and flours and the values obtained are shown in OSM1. On a dry weight basis, the measured total starch (TS) content was slightly higher for pulse powders (range from 40 to 60 g TS/100 g DM) than flours (range from 30 to 49 g TS/100 g DM), presumably due to the loss of soluble components during preparation of the powders. However, unlike cooked pulse flours (Fig. 6A) the starch in the PulseON® powders was highly resistant to digestion, as observed in the starch digestibility profiles shown in Fig. 6B. Less than 40% starch in the PulseON® had been digested after 90 min, whereas cooked pulse flours were rapidly digested (> 80% starch digested within 30 min). This was true for all the botanical sources of pulses tested. It is noteworthy that the starch digestibility of BB flour appears in Fig. 6A to exceed 100%; however, the authors believe that inaccuracies in starch determination methods, which are frequently associated with 10–20% errors (according to the information provided by the test-kit supplier, Megazyme International Ltd., Ireland) has led to an underestimation of the starch content of BB in this case. Nevertheless, all gelatinised flours were clearly highly digestible, with negligible amounts of resistant starch, compared to PulseON® powders.

Out of all the PulseON® powders, the ingredient prepared from chickpeas was found to have one of the highest starch digestibility profiles, and so this was chosen for the Glycaemic Index (GI) study in human participants. Incremental postprandial glucose response profiles obtained from the reference (glucose) and PulseON® test drinks is shown in Fig. 6C together with the calculated GI values for chickpea PulseON® (Fig. 6D). When calculated for each individual, the GI mean and SD was 63 ± 37, however, three participants displayed aberrant OGTT responses (GI > 100, due to minimal glycaemic response to the OGTT and to the test meal) which skewed the data. Excluding those three outliers resulted in a mean GI of 48 ± 16.5.

4. Discussion

The aim of the study was to characterise novel pulse powders obtained from seven different botanical sources and compare their properties with flours obtained from the same source. We have shown that the alternative processing method used resulted in cellular powders

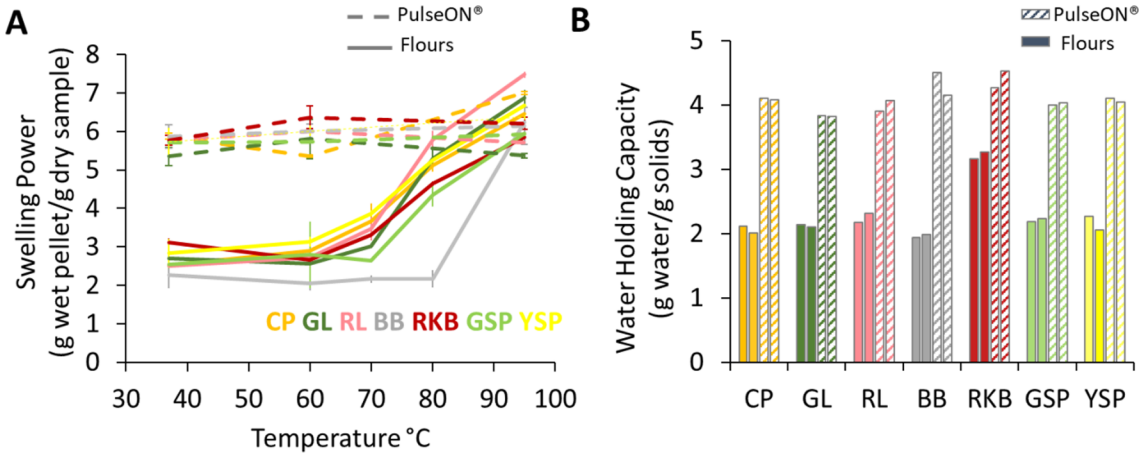

Fig. 4. Swelling power (A) and water holding capacity (B) of flours and PulseON® powders. Values for swelling power are means of powder (n = 2) and flour (n = 3), and for water holding capacity, measured at 20 °C, values are shown as duplicates. Materials were obtained from the following pulses; chickpea (CP), green lentils (GL), red lentils (RL), butterbeans (BB), red kidney beans (RKB), green split peas (GSP), and yellow split peas (YSP). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Enthalpy values, associated with the lower temperature endotherms, for pulse flours and PulseON® powders.[1]

		T_o (°C)	T_p (°C)	T_e (°C)	ΔH (J/g)
Flours	CP*	64.8 ± 0.2	71.9 ± 0.0	82.0 ± 0.2	5.6 ± 0.1
	GL	62.7 ± 0.0	71.4 ± 0.2	82.3 ± 0.0	6.8 ± 0.1
	RL*	61.2 ± 0.2	69.9 ± 0.0	80.6 ± 1.9	5.8 ± 2.2
	BB*	80.3 ± 0.0	86.1 ± 0.1	96.8 ± 0.3	9.0 ± 0.7
	RKB*	62.1 ± 0.4	73.6 ± 0.0	82.5 ± 0.2	3.6 ± 0.3
	GSP	64.0 ± 0.5	74.1 ± 0.1	85.4 ± 0.7	7.8 ± 0.0
	YSP	61.2 ± 0.2	69.4 ± 0.0	80.7 ± 0.4	6.3 ± 0.7
PulseON®	CP*	52.1 ± 0.3	60.1 ± 0.0	70.6 ± 0.4	2.9 ± 0.3
	GL	52.1 ± 0.3	61.1 ± 0.3	74.0 ± 0.4	3.6 ± 0.1
	RL	51.8 ± 0.7	60.2 ± 0.6	72.2 ± 0.2	3.5 ± 0.3
	BB	50.9 ± 0.2	64.3 ± 0.1	79.0 ± 0.1	3.3 ± 0.1
	RKB	52.0 ± 0.3	60.6 ± 0.3	72.4 ± 0.5	3.3 ± 0.3
	GSP*	52.6 ± 0.1	61.5 ± 0.0	73.9 ± 0.1	4.1 ± 0.0
	YSP*	52.0 ± 0.1	61.3 ± 0.5	72.7 ± 0.2	4.7 ± 0.1

[1] Mean values with standard error were obtained from duplicate DSC runs of samples heated in water (liquid to solids ratio of 3:1) from 20 to 150 °C at a rate of 10 °C/min, with onset T_o, peak T_p and concluding T_c temperature and gelatinisation enthalpy ΔH (J/g sample) obtained from the DSC endotherms. Materials were obtained from the following pulses; chickpea (CP), green lentils (GL), red lentils (RL), butterbeans (BB), red kidney beans (RKB), green split peas (GSP), and yellow split peas (YSP).

* Denotes an observable second endothermic peak with a peak temperature in the range of 80–100 °C.

(trademarked PulseON®) containing starch that is highly resistant to amylase digestion and with demonstrably low to medium Glycaemic Index values. These signature characteristics of PulseON® were present regardless of the type of pulse used to make the powder. The characterisation tests performed on these novel materials revealed differences between flour and cell powders that are of relevance to product formulation. Thus, PulseON® powders are novel and versatile functional food ingredients that can be prepared from a number of different sources and added to foods so that consumers can benefit from the nutritional properties of pulses.

The pulse powders consisted almost exclusively of intact, individual starch-filled plant cells, which were harvested through alternative processing methods from the cotyledonous tissue of each seed. Thus, a critical feature that distinguishes PulseON® from conventional dry-milled flour is its cellular integrity, which ensured that starch remains encapsulated by the plant cell wall, i.e. as Type 1 resistant starch. The starch within pre-cooked PulseON® powders was found to be highly resistant to digestion, with less than 40% starch becoming digested after 90 min incubation with α-amylase. Starch digestibility of cooked dry-milled flour on the other hand was > 70–80% after 90 min incubation, representing the maximum extent of digestion achieved by amylolysis of starch. Similarly, the integrity of the cell wall in PulseON® is demonstrated by the stability of the swelling power with increasing temperature, particularly above 80 °C, which exceeds the gelatinisation temperature of starch.

We recently reported a reasonable correlation between the *in vitro* starch digestibility screening method and GI values obtained from published literature values (Edwards, Cochetel et al., 2019), and predicted from the C90 values that these ingredients would evoke low postprandial glycaemic responses *in vivo*. The human study confirmed the glycaemic response to chickpea PulseON®; on the basis of the average GI measured for chickpea PulseON® *in vivo*, the ingredient would be classed as a medium glycaemic index agent (i.e. having a GI between 55 and 70), it is noteworthy that the distribution was skewed by three values outside the 3rd quartile, and for 10 out of the 18 subjects, PulseOn® had a GI of less than 55. When the three outliers were excluded, the mean (n = 15) GI was 48. Thus, the term low to medium glycaemic index ingredient more justifiably describes one of its nutritional properties.

Mechanistically, we consider that the attenuated glycaemic response and high degree of starch resistance can probably be attributed to the structurally intact cell walls (i.e. encapsulating dietary fibre), which has been reported to delay/hinder ingress of digestive enzymes (Bhattarai et al., 2017; Dhital et al., 2016; Rovalino-Córdova, Fogliano, & Capuano, 2019). This finding is consistent with observations of limited starch digestibility from materials containing high proportions of intact cotyledon cells in pulses (Berg et al., 2012; Edwards et al., 2014, 2018; Würsch et al., 1986).

The similar characteristics obtained regardless of the type of pulse used is consistent with our expectation that this new technology can be applied to a range of different legumes. Interestingly, different pulses produced powder with different colours (visible to the naked eye).

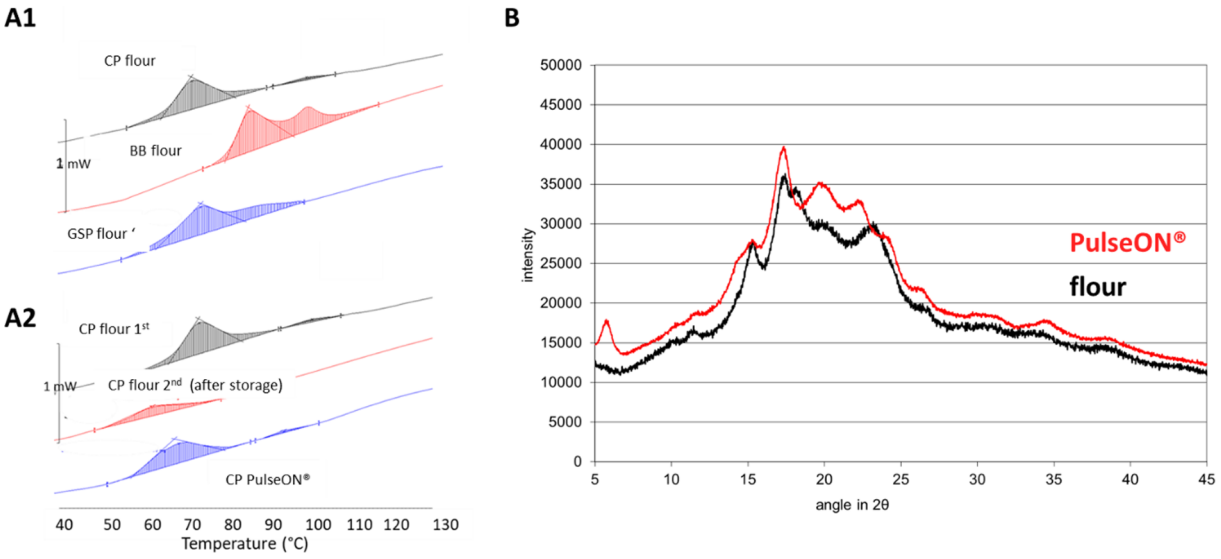

Fig. 5. Enthalpy (A) and crystallinity (B) of flours and PulseON® powders. Example DSC endotherms obtained on first heating of flours from chickpea (CP), butterbean (BB) and green-split pea (GSP) are shown in A1. Panel A2 shows an endotherm obtained for CP PulseON® alongside endotherms obtained on first heating of raw chickpea flour (annotated '*CP flour 1st*') and for re-heated CP flour after storage at 4 °C for 7 days (annotated '*CP flour 2nd*'). All DSC endotherms were obtained using a ratio of water to solids of 1:3 and heating from 20 to 150 °C at a rate of 10 °C/min. X-ray patterns for chickpea flour and corresponding PulseON® powder are shown in panel B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

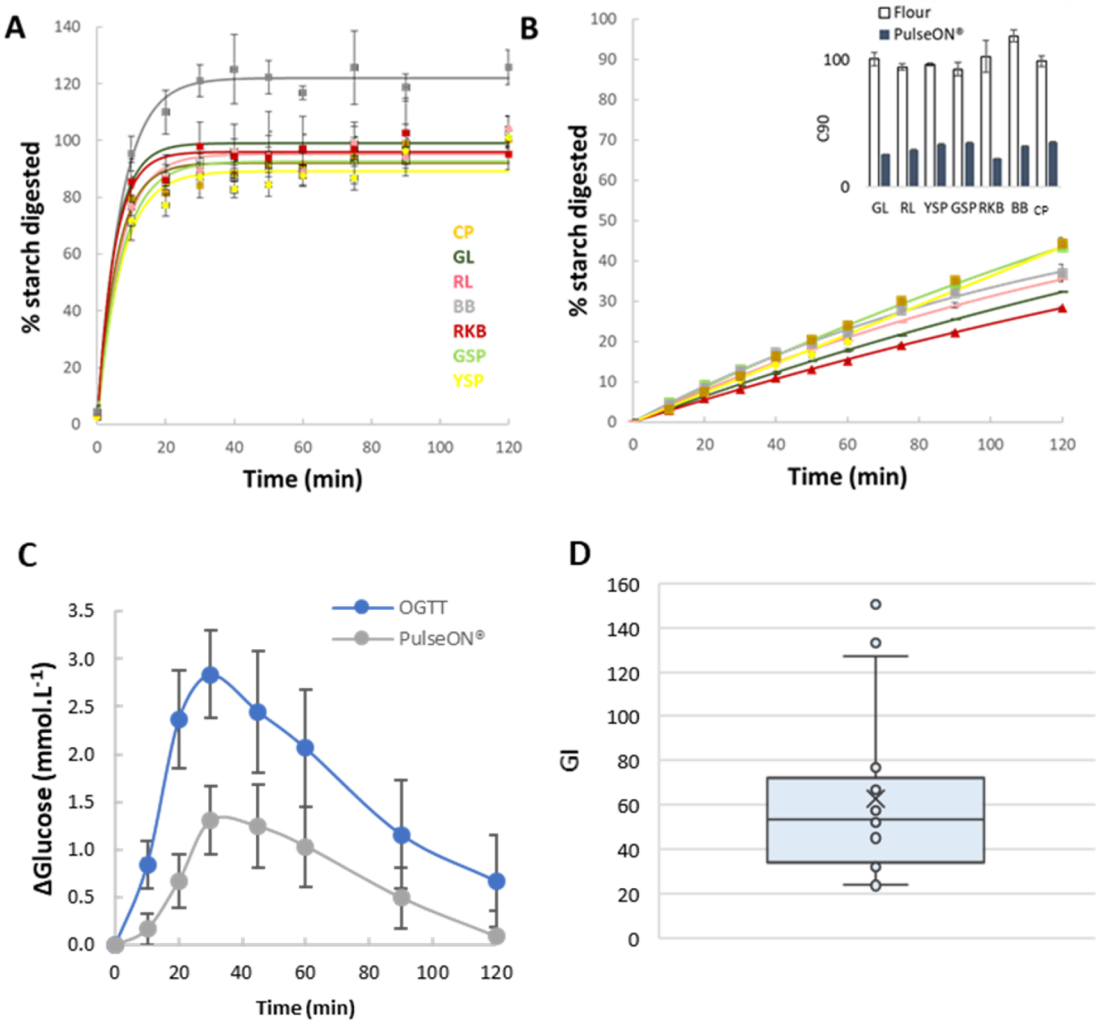

Fig. 6. *In vitro* starch digestibility (AB) and *in vivo* glycaemic responses (CD). Individual time points of the starch digestibility curves of gelatinised flour (A) and PulseON® powders (B) are shown as means of triplicate analysis with SEM, and with C90 values shown as an insert (B). Data in A and B were obtained from various pulses; chickpea (CP), green lentils (GL), red lentils (RL), butterbeans (BB), red kidney beans (RKB), green split peas (GSP), and yellow split peas (YSP) and the legend applies to both panels. Acute postprandial glycaemic responses to Chickpea PulseON® compared with isoglucidic reference drink 'OGTT' are shown in (C) as mean incremental (Δ) glucose concentrations in capillary blood samples measured in 18 healthy subjects with error bars as 95% confidence intervals. The Glycaemic Index, 'GI', of chickpea PulseON® determined for all subjects (n = 18) is shown as a box-and-whisker plot (D), in which mean GI = 62.9, lower quartile = 36.9, median = 53.5, and upper quartile = 70.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PulseON® powders prepared from pulses with dark coloured testa (GL and RKB) were stained by leakage of pigments (proanthocyanidins) from the testas during cooking, whereas the colour of lentil powders may depend on the carotenoid concentration. Chlorophyll and xanthophylls may be responsible for the green and yellow colours, respectively, of peas, which was more strongly observed in the flour than the powder. Overall, this natural diversity provides opportunities to produce ingredients that match the desired colour profile in the final product. Processing peas into PulseON® also overcomes the green colour and flavour of pea flour, which is an undesirable sensory characteristic for many product applications.

In terms of yield, all seven pulses provided a similar quantity of cellular PulseON® powder. Considering that only a proportion of cells within each cotyledon are of a type that have a tendency to cell separate, and taking account of the relative proportions of the embryo (1%) and testa (5%), we estimated that the maximum theoretical yield of cell powder was 55%, with losses of soluble components to aquafaba (5%), abaxial cell layers (30%), and to cooking (3%). The yields obtained were therefore reasonably close to the theoretical maximum. For commercial purposes, the waste streams can be re-incorporated or valorised, for instance, the testa provide a useful source of insoluble fibre, while aquafaba is used as a vegan egg-white mimic (Buhl, Christensen, & Hammershøj, 2019).

There were important differences between flour and cell powder which have implications for their use in product formulation. PulseON® is a pre-cooked powder and is more thermally stable than raw flour. The novel ingredient was found to bind more water at 20 °C than raw pulse flour and maintained a constant swelling power over a range of temperatures. The results from the DSC studies suggest that the cellular powders contain some retrograded and ordered starch that undergoes an endothermic transition at a lower temperature than the raw, dry-milled flours from the same botanical source. Most of the pulses tested had similar properties although BB swelled and gelatinised at a higher temperature than the other pulse flours. In all the characterisation tests performed, the processing treatment applied to obtain pulse flour or PulseON® had a far greater impact on material characteristics than the botanical source. These physical properties are important for producing the textures and other quality attributes of food products. Although PulseON® powders had a similar appearance to dry-milled flour, they will not mimic the functional characteristics of flour. The data provided

in the present study will therefore provide valuable insight to guide future product development.

Legume powders containing cells have been described by other groups previously. Tosh *et al.*, described spray-dried legume powders with ~5% resistant starch (Tosh et al., 2013); however, when tested in acute human dietary intervention studies these spray-dried and milled legume powders did not retain the low postprandial glycaemic responses of the original legumes (Ramdath et al., 2018). Also, the legume flours had no effects on cardiovascular disease risk or glycaemic control when administered over 28 days (Cryne et al., 2012). A study by Boukid *et al.* reported promising effects of pulse flours containing intact cells in gluten free bread, however the cells in this study were present as clusters within macro-particles consisting of intact and ruptured cells, rather than as intact isolated cells that are characteristic of PulseON® (Boukid et al., 2019). The water holding capacity of pulse powders described in Boukid *et al.* were similar to those observed for pulse flour within the present study, reflecting the different structures of these ingredients. Owing to the differences between PulseON® and previously described legume powders, further human studies will be required to ascertain the effects of this unique powder on various health outcome measures.

Pulse powders are already used in the gluten-free market but could bring nutritional advantages into mainstream food products. For instance, PulseON® contains approximately twice as much protein and fibre as that found in wheat flour, yet only half the starch content. Thus, replacing wheat flour in a product with PulseON® would lower its glycaemic impact and provide a complementary lysine-rich protein source. Furthermore, our finding that the starch within PulseON® is Type 1 resistant starch with a low-medium glycaemic index is promising for the development of future foods to benefit gut health and glycaemic control, which may in turn have a positive impact on cardiometabolic health, and reduce the risk of cardiovascular disease and type 2 diabetes (Augustin et al., 2015; Levitan, Song, Ford, & Liu, 2004; Sievenpiper et al., 2009). It is noteworthy that resistant starch is not necessarily thermally stable and the digestibility of any starch-rich ingredient will be susceptible to change during secondary processing (Wang & Copeland, 2013). Further work is therefore needed to assess the compatibility of PulseON® with different product categories, and to determine its bioefficacy when administered as part of a realistic food product.

5. Conclusions

Peas, chickpeas, lentils and beans were processed into PulseON® cell powders and their characteristics compared to dry-milled flours obtained from the same botanical sources. Substituting pulse flour with PulseON® provided similar nutritional composition, but with Type 1 resistant starch that was considerably more resistant to digestion and therefore elicited a lower glycaemic response. The functional characteristics of PulseON® powders differed from pulse flours, and the characteristics reported here are important considerations for successful product re-formulation. Finally, the differences between botanical sources were negligible compared to the large differences achieved through alternative processing methods, and this work serves as an example of how targeted processing can be used as a powerful tool for making major nutritional improvements to existing crops.

CRediT authorship contribution statement

Cathrina H. Edwards: Conceptualisation, Supervision, Project administration, Visualization, Writing - original draft, Writing - review & editing, Funding acquisition. **Peter Ryden:** Investigation, Data curation, Formal analysis. **Ana M. Pinto:** Investigation, Data curation, Formal analysis. **Alice van der Schoot:** Investigation, Data curation, Formal analysis. **Costanza Stocchi:** Investigation, Data curation, Formal analysis. **Natalia Perez-Moral:** Investigation, Data curation, Formal analysis. **Peter J. Butterworth:** Methodology, Writing - review & editing. **Balazs Bajka:** Conceptualisation, Project administration, Investigation, Supervision; Writing - review & editing. **Sarah E. Berry:** Methodology, Supervision. **Sandra E. Hill:** Resources, Investigation, Supervision. **Peter R. Ellis:** Supervision, Writing - review & editing.

6. Ethics statement

The authors confirm that the work described involving humans has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and that all human participants gave full written informed consent before participating in this research.

Declaration of Competing Interest

There is no obvious immediate conflict of interest. The PulseON® material is covered by a published patent application but this ingredient product is not commercialised. The patent is authored by four of the authors of this paper, namely CHE, PJB, SEH and PRE.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.103918.

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